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# Crown Infection of Corn by *Diplodia zeae*

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BOTANY AND PLANT PATHOLOGY SECTION



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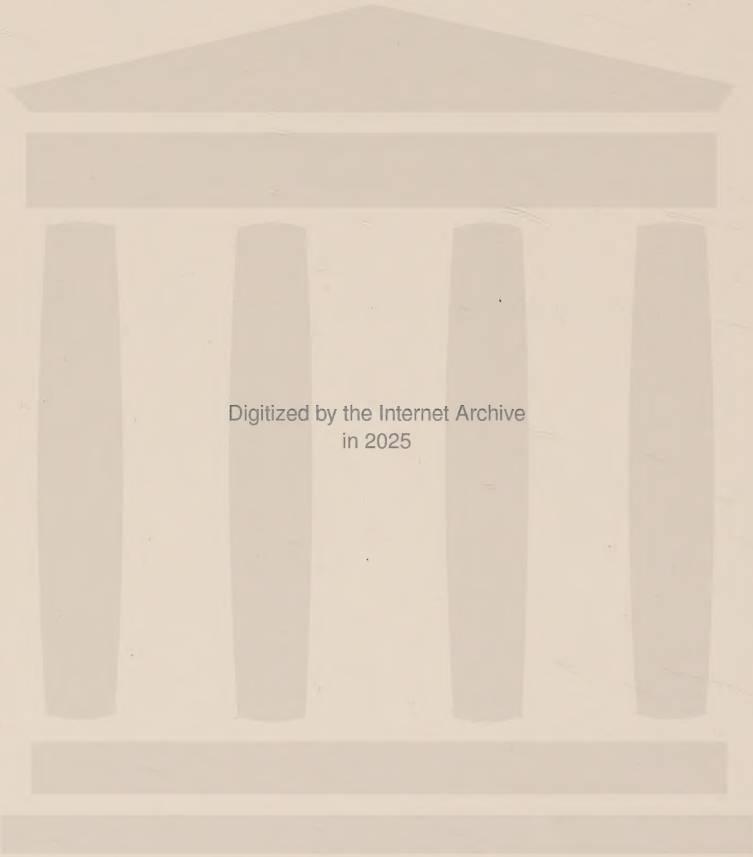
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## CONTENTS

|                                                                                                    | Page |
|----------------------------------------------------------------------------------------------------|------|
| Summary .....                                                                                      | 191  |
| The source of inoculum and nature of crown infection.....                                          | 194  |
| Symptoms of crown infection .....                                                                  | 194  |
| Prevalence of crown infection .....                                                                | 197  |
| Source of inoculum .....                                                                           | 198  |
| Invasion and progress of infection in the crown.....                                               | 201  |
| Effect of seed treatment on late crown infection.....                                              | 203  |
| Factors influencing crown invasion from infested soil and its<br>effect upon the host .....        | 205  |
| Effect of soil moisture upon the saprophytic existence of<br><i>Diplodia Zeae</i> in the soil..... | 205  |
| Effect of soil moisture upon crown invasion from infested<br>soil .....                            | 206  |
| Methods .....                                                                                      | 207  |
| Preliminary experiments .....                                                                      | 207  |
| Experiment of 1932 .....                                                                           | 209  |
| Experiment of 1933 .....                                                                           | 210  |
| Reduction in development of the plant by crown infection                                           | 211  |
| Effect of crown infection on the transpiration ratio.....                                          | 215  |
| Reaction of selfed lines to late crown infection.....                                              | 215  |
| Discussion .....                                                                                   | 217  |
| Literature cited .....                                                                             | 221  |



## SUMMARY

The symptoms of crown infection of corn caused by *Diplodia zeae* include: a dark straw-brown discoloration of the tissues of the crown and lower internodes; prevalence of subepidermal pycnidia on the crown and around the aerial adventitious roots; disintegration and shredding of the internal tissue of the crown; intense brown discoloration of the nodal plates, and a dark brown decay of the mesocotyl which results in the loss of the primary root system.

Crown infection occurred on 14 to 52 percent of the field corn in central Iowa in the years from 1930 to 1933.

Practically every corn plant grown from *Diplodia*-infected seed may show crown infection at maturity. Infected seedlings which, by means of timely establishment of adventitious roots, do not die may be parasitized during the remainder of the growing season.

The symptoms on plants grown from disease-free seed in *Diplodia*-infested soil are identical with those derived from infected seed. The pathogene grows through the soil to the mesocotyl where it becomes established in the wounds created by the emerging seminal roots. The crown may be invaded directly from the soil at the point of emergence of an adventitious root but the crown is usually invaded from the decayed mesocotyl.

The pathogene progresses up the mesocotyl from this primary lesion and becomes established on the crown. The mycelium spreads internally to a limited extent during the growing season and rapidly involves the entire crown and lower nodes at maturity.

Although *Diplodia zeae* spreads from the mesocotyl upward for several nodes, it is not systemic in the plant. Under field conditions it is generally restricted to the first internode above the roots.

Crown infection has been induced by infesting steamed soil with infected crowns and stalks of plants which had over-wintered under field conditions.

*Diplodia zeae* may live in a soil devoid of plant refuse but its development is hampered in mixed culture. The presence of the pathogene in ordinary soil from a corn field has been demonstrated by growing plants from disease-free seed in such soil collected in the spring.

Invasion of the crown from infested soil is most severe at high soil moisture contents, but the development of the infected plants is severely reduced at either high or low moisture contents. The dry weight of plants growing at optimum soil moisture content was not reduced by crown infection.

The dry weight of roots is reduced more than that of the tops of plants grown in infested soil. The dry weight of the tops, however, is significantly less than that of non-infected plants grown under soil moisture conditions approaching the maximum or minimum for growth.

A light infection of a plant growing at low soil moisture may be almost as injurious to the plant as a severe infection where there is an abundant supply of water.

The transpiration ratio of infected plants is increased at soil moisture contents favorable to reduction in dry weight.

Plants grown from *Diplodia*-infected seed treated with a mercury dust had a higher percentage of crown infection than similar plants from untreated seed. Treatment of disease-free seed with the same dust decreased the amount of crown infection of plants growing under field conditions. The dust apparently inhibited the fungus without killing it in the seed. Plants protected in this fashion later suffer crown infection.

The extent of crown invasion under given conditions depends upon the time of initial infection. Seed treatment of disease-free seed may delay mesocotyl invasion from infested soil and thereby decrease crown infection.

Selfed lines of corn have different reactions to crown infection by *Diplodia zeae* under field conditions and furnish some remarkably resistant lines for genetical studies.

Late crown infection is a significant phase of the parasitism of *Diplodia zeae* on corn. The pathogene may reduce the dry weight of the plant to half that of a normal plant under certain soil moisture conditions. Under the most favorable field conditions there is a reduction in yield.



# Crown Infection of Corn by *Diplodia zeae*<sup>1</sup>

BY GEORGE L. MCNEW<sup>2</sup>

The pathogenicity of *Diplodia zeae* (Schw.) Lév.<sup>3</sup> on the corn plant during the ear and seedling stages has been thoroughly described in the contributions of Burrill and Barrett (3), Durrell (5), Holbert *et al.* (7), Manns and Adams (16, 17), Raleigh (22) and others. The activity of *Diplodia zeae* on the host between the seedling and ear stages, however, has received relatively little consideration. Durrell (5) discussed the prevalence of *Diplodia zeae* on the crown and lower nodes of corn plants, but attributed the unusual amount of infection in these points to the favorable conditions for infection by wind-blown spores rather than to invasion from previously infected underground parts.

The crown and basal portions of the stalk, however, might be invaded by mycelium of *Diplodia zeae* from infected seed or infested soil, as well as from wind-borne spores. Since the infection on the mesocotyl of plants which do not die as seedlings has generally been considered of little importance, the exact relationship between seedling and crown infection has never been adequately studied. Koehler and Holbert (15) expressed what seemed to be a generally accepted opinion in 1930 as follows: "... the fungus attack (from infected seed) is local, beneath the soil surface, and after the infected parts of the seedling have decayed the activity of the fungus in that region probably soon comes to an end."

Data are presented below which tend to show that *Diplodia zeae* does not cease its parasitic development once the mesocotyl has been infected, even though an adventitious root system is established. The pathogene spreads from the mesocotyl up into the crown, especially late in the growing season. Where *Diplodia zeae* is present in the soil on corn refuse from a preceding crop, it may attack the mesocotyl and later spread up into the crown in the same manner as when mesocotyl infection is derived from infected seed. The studies reported in this bulletin deal with the prevalence of crown infection, its effect upon the corn plant, and the influence of certain

<sup>1</sup>Project 93 of the Iowa Agricultural Experiment Station. This bulletin constitutes one section of a thesis submitted in partial fulfillment of the requirements for the degree of doctor of philosophy.

<sup>2</sup>The author expresses appreciation to Dr. I. E. Melhus for proposing the problem, offering timely suggestions during the course of experimentation and constructively criticising the manuscript. Thanks are also due Dr. C. S. Reddy for many helpful hints and permission to examine corn plants in his seed corn treatment plots.

<sup>3</sup>In view of its widespread use, the specific name *Diplodia zeae* has been retained in preference to *D. maydis* (Berk.) Sacc. This suggestion has been advanced by Shear and Stevens (25) who have discussed the confusion in nomenclature of this fungus.



Fig. 1. Crown infection on sweet corn plant, Golden Bantam variety, grown from *Diplodia*-infected seed. The plant on the left, grown in steamed compost from an infected seed died 90 days after emergence while plants, such as the one on the right, grown from non-infected seed under identical conditions did not mature until 120 days. As a general rule crown infection hastens maturity only 1 or 2 weeks. Note the discolored crown and stalk of the infected plant which support pycnidia of *Diplodia zeae*.

environmental factors on the growth and pathogenicity of *Diplodia zeae* in the soil.

#### THE SOURCE OF INOCULUM AND NATURE OF CROWN INFECTION

The term crown infection has been applied to the invasion of the crown and the lower internodes either above or below the aerial adventitious roots. Although the fungus usually invades the crown from previously infected underground parts, the most characteristic symptoms appear just previous to or at maturity.

#### SYMPTOMS OF CROWN INFECTION

Infected plants are frequently subnormal in development and may mature 1 to 2 weeks before non-infected plants (fig. 1). As the crown and lower internodes become dry, they assume a dark straw-brown color in contrast to the light color of normal plants (figs. 1, 3). This discoloration may extend as a definite lesion between the roots, but it is usually more diffuse, involving the entire crown and lower internodes.

The lesions on the mature host may be distinguished from other crown injuries by the numerous minute, black pycnidia of *Diplodia*



*zeae*. The pycnidia appear first as light colored specks, which soon become black subepidermal bodies. These pycnidia may extrude long black spore tendrils by late October or November if the soil supporting the plant is wet. Some infected plants may not have pycnidia, particularly when infection occurs late in the plant's life history.

When infected crowns are split open the pith is found to be disintegrated, leaving the vascular bundles conspicuously separated and exposed. The nodal plates at the base of the crown are partially digested and dark brown in color (fig. 3). Wefts of mycelium are very conspicuous in the pith cavity of infected tissues.

Although pycnidia may be found on the larger adventitious roots near the crowns of infected plants, invasion of the roots is not very common under field conditions. Pronounced lesions may develop directly on roots of plants grown under greenhouse conditions in compost heavily infested with *Diplodia zeae*, (fig. 2), but similar lesions have not been observed in the field.

The initial symptom of late crown infection usually appears on the mesocotyl during the seedling stage. When the fungus is seed-borne it gains entrance into the wounds created by the emergence

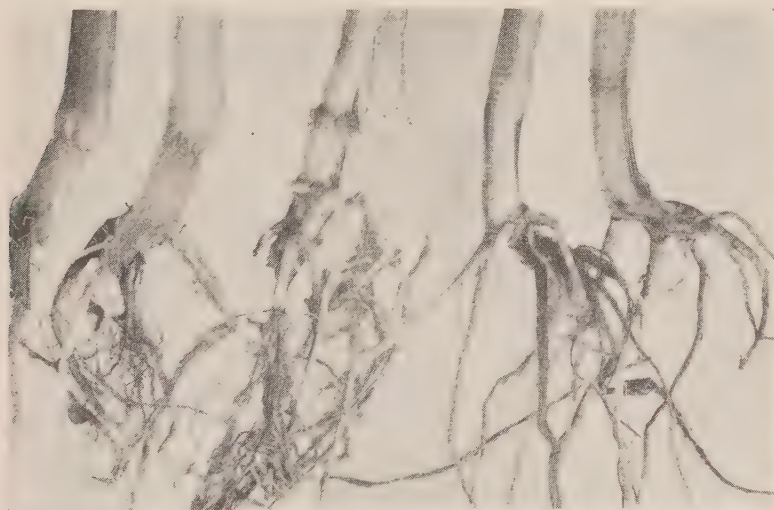


Fig. 2. Pathological symptoms of plants infected with *Diplodia zeae* immediately preceding maturity. All the plants were grown from disease-free seed. The plant at the left was grown in steamed compost while the other two were grown in similar compost infested with *Diplodia zeae*. Note the clear white mesocotyl and crown of plant at the left. The mesocotyl and primary root system of corn remain functional until maturity if not infected. The plant in the center illustrates direct crown invasion through the wound created by the emergence of a secondary root and the plant at the right illustrates the more frequent invasion from the infected mesocotyl. The lesion has extended through the crown and into the first node although the exterior of the plant is injured very slightly.

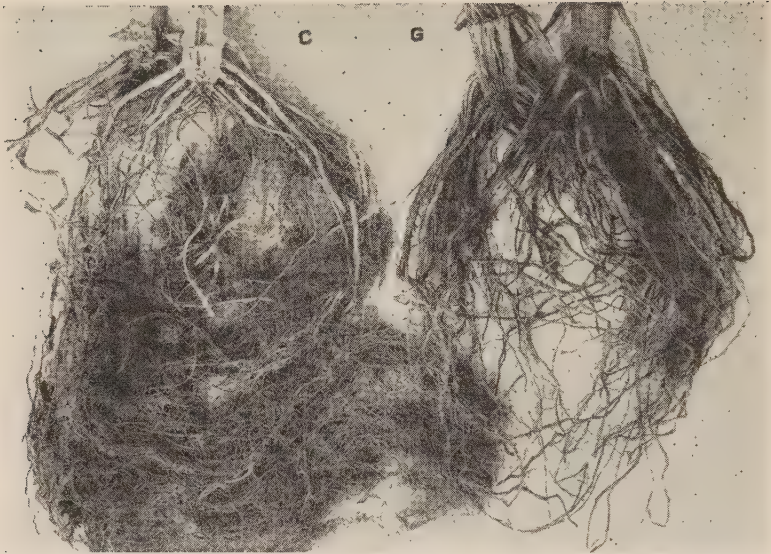


Fig. 3. The effect of late crown infection on field corn. These two plants were grown side by side for 106 days in large iron cans containing greenhouse compost adjusted to 55 percent of the water-holding capacity. The soil supporting "G" was infested with *Diplodia zeae* on steamed oats during the seedling stage, while steamed oats alone was worked into the compost supporting "C." Note the difference in external and internal coloration of the crown tissue and roots, the minute pycnidia and internal shredded appearance of "G."

The status of the two plants at maturity was:

|                     | "C"      | "G"     |
|---------------------|----------|---------|
| Dry weight of roots | 190 gm.. | 70 gm.  |
| Dry weight of top   | 384 gm.  | 323 gm. |
| Transpiration ratio | 149      | 192     |

of the seminal roots. Raleigh (22) has adequately described these symptoms for seedling blight. However, those seedlings which establish adventitious roots before the mesocotyl is completely girdled continue to grow. The dark brown, sunken lesion is extended until the entire mesocotyl is involved, usually several weeks after emergence of the seedling. *Diplodia zeae* growing from infested soil usually becomes established in the wounds at the base of the mesocotyl. However, infection may occur farther up the mesocotyl or at the wound caused by the emergence of an adventitious root from the crown.

In either case, the fungus establishes itself on the base of the crown by midseason, but does not appear immediately to advance farther. Later the lesion may be extended upward between the adventitious roots, but does not involve the entire crown nor extend above ground until the plant begins to mature. Although there is little evidence of extensive external injury during this period, there may be a progressive discoloration of the xylem and outer parenchymatous cells.



## PREVALENCE OF CROWN INFECTION

Plants in representative fields near Ames, Iowa, were pulled, the soil removed from the crowns and the amount of infection recorded for the years 1930 to 1933, inclusive. Discoloration of the crown and the presence of pycnidia in the lesions were used as the diagnostic symptoms. When discoloration and distinct lesions occurred without conspicuous pycnidia, isolations were made in 1930 and 1931 to establish the causal relationship. In such cases, all soil was washed from the crown which was cut into pieces and surface sterilized for 45 seconds in mercuric chloride (1 to 1000), washed in sterilized distilled water and planted on corn meal agar. *Diplodia zeae*, *Gibberella saubinetii* (Mont.) Sacc., *Fusarium moniliforme* Sheld., and species of *Trichoderma*, *Penicillium* and *Aspergillus* were isolated.

Less than 1 percent of the 500 plants examined in 20 different fields during September, 1930, had pycnidia of *Diplodia zeae* on the crowns; however, *Diplodia zeae* was identified from 10.8 percent of the crowns through isolation studies. Another survey made in late October showed 13.9 percent of the plants to have typical late crown infection in which pycnidia were present.

These data in 1930 indicated that readings should be delayed until the plants are fully mature; therefore, the subsequent field surveys in 1931, 1932 and 1933 were taken in October and November to insure that the reading would include all infected plants. Since such a delay might allow time for invasion after the plant had died, several tests were made to determine if diagnostic symptoms would be produced by saprophytic invasion of mature normal tissue. Disease-free plants were selected from the field and allowed to dry out thoroughly. The stalks were cut into several pieces and placed in contact with cultures of *Diplodia zeae* in steamed moist soil and in sand. After 3 weeks the pith was slightly affected; but the subepidermal pycnidia, used as the basis of readings presented in table 1, were absent. On two different occasions sets of plants were grown to full maturity from disease-free seed in steamed compost and allowed to dry naturally. A culture of *Diplodia zeae* on steamed oats was worked into the soil around the roots of the dead plants. After 3 or 4 weeks the soil was washed from the roots and the plants were examined. Pycnidia appeared on only 1 out of 200 plants and were confined to the area beneath the aerial adventitious roots. Although diagnostic symptoms were absent, several of the plants were partially decayed at the soil line, and mycelium was very common in the pith cavity.

The percentages of crown infection, presented in table 1, varied widely in the different years under consideration. A higher percentage of crown infection was recorded in the wet year of 1932 than in the abnormally dry season of 1930. These readings represent the minimum amount of infection, since they are based entirely

TABLE 1. THE PREVALENCE OF *DIPLODIA ZEA* ON THE CROWN AND LOWEST INTERNODE OF CORN PLANTS IN THE VICINITY OF AMES, IOWA

| Date of survey | No. of fields examined | No. of plants counted | Percentage of plants with <i>Diplodia zeae</i> pycnidia | Percentage of infected plants without pycnidia* |
|----------------|------------------------|-----------------------|---------------------------------------------------------|-------------------------------------------------|
| Sept. 1930     | 20                     | 550                   | 3.9                                                     | 10.8                                            |
| Oct. 1930      | 1                      | 774                   | 13.9                                                    | ---                                             |
| Oct. 15, 1931  | 11                     | 236                   | 27.5                                                    | 14.6                                            |
| Oct. 20, 1932  | 2                      | 1298                  | 52.4                                                    | ---                                             |
| Nov. 20, 1933  | 10                     | 1698                  | 23.2                                                    | ---                                             |

\*Determined by plating tissue from crowns on agar plates.

upon the presence of pycnidia. In 1931 one-third of the amount of infection was missed in such readings, as was shown through the isolation studies.

#### SOURCE OF INOCULUM

Nearly-disease-free and *Diplodia*-infected seed were planted in adjacent blocks at the rate of two plants per hill in 1930 and 1931. Corn had been grown in the fields the preceding years. The percentages of crown infection, in October and November, based upon production of pycnidia, are presented in table 2.

These data indicate that a large proportion of crown infection was derived from aerial or soil-borne inoculum. However, the larger percentage of infection of plants from infected seed, the difference being significant in 1931, would suggest that at least a part of the infection under field conditions may be derived from seed.

Belief in the ability of the seed-borne mycelium to induce late crown infection was substantiated by following the course of infection in plants grown from infected seed in steamed compost under greenhouse conditions. From 200 seeds planted a stand of 63 percent was obtained, and 13 percent of the emerged seedlings were dead within 3 weeks. When 80 of the remaining plants were removed and examined, 16 percent were found to have mesocotyls free of lesions. Four weeks after emergence of the seedlings the soil in half of the remaining 30 plant cultures was infested with *Diplodia zeae* growing on whole steamed oats. At maturity the plants were examined for characteristic lesions, and pieces of tissue from the various sections of the crown and stem were planted on corn meal agar. These data, presented in table 3, show that practically every infected plant which did not die as a seedling suffered from crown infection at maturity. There was little increase in the amount

TABLE 2. THE PERCENTAGE OF CROWN INFECTION ON PLANTS DERIVED FROM NEARLY-DISEASE-FREE AND *DIPLODIA*-INFECTED SEED.

| Character of seed used    | Date of observation |                     |                |                     |
|---------------------------|---------------------|---------------------|----------------|---------------------|
|                           | October, 1930       |                     | November, 1931 |                     |
|                           | No. plants          | Percentage infected | No. plants     | Percentage infected |
| Nearly-disease-free       | 217                 | 12.4                | 200            | 62.5                |
| <i>Diplodia</i> -infected | 160                 | 16.9                | 142            | 88.2                |

TABLE 3. NUMBER OF PLANTS INFECTED WHEN CORN WAS GROWN FROM DIPLODIA-INFESTED SEED IN STEAMED AND DIPLODIA-INFESTED SOIL.

| Treatment of compost | No. plants* with lesions |      | Diplodia isolated from |                 |                  | No. plants with pycnidia |
|----------------------|--------------------------|------|------------------------|-----------------|------------------|--------------------------|
|                      | Crown                    | Stem | Crown                  | First internode | Second internode |                          |
| Steamed              | 13                       | 12   | 10                     | 10              | 8                | 4                        |
| Infested             | 14                       | 13   | 12                     | 12              | 9                | 7                        |

\*Based upon 15 plants in each group.

of infection where the soil was infested, since practically all of the plants were already infected from the seed. Disease-free seed, planted in the same non-infested soil and held in the same greenhouse, produced apparently healthy plants.

A repetition of this experiment gave comparable results. Where infected seed was used, late crown infection appeared at maturity. Such delayed infection might easily have been overlooked by those who have accepted the idea that the fungus in the mesocotyl desists after the seedling stage. As stated by Clayton (4), Durrell (5), Vibar (27) and others, infected seedlings are never killed after the adventitious roots are established. Under field conditions, therefore, the first conspicuous symptoms would appear at maturity. Since the plants would not be grown under rigidly controlled conditions the logical assumption would be that infection had been induced by wind-borne spores lodging in the lower leaf sheaths as described by Durrell (5). Those studying seedling infection would probably discard the plants grown under greenhouse conditions before such infection became conspicuous.

Disease-free seed of dent and sweet corn was planted in sterilized compost. Each container was infested with *Diplodia zeae* growing on steamed oats at different periods after emergence of the seedlings. The sweet corn plants were grown to maturity, and the soil in half the pots was infested with an oat culture of *Diplodia zeae* at 90 days after emergence. The soil was washed from the roots of the sweet corn after 110 days and from the field corn after 130 days. The plants were then examined for lesions and pycnidia of *Diplodia zeae*. Isolations were made from fragments of the crowns placed on corn meal agar. The data on infection, based upon the presence of lesions and isolation of the fungus from the crown and stalk on corn meal agar in these two series are presented in table 4.

On the sweet corn plants the fungus progressed farther up the stalk when the soil was infested early in the plant's development, but the trend easily may have been an accident of sampling. Reinfestation of the soil at maturity of the plant did not appreciably increase the severity of infection. Field corn and sweet corn were infected with equal ease. Every plant growing in soil infested immediately after emergence suffered crown infection. These results were confirmed by repetition of the experiments.



TABLE 4. NUMBER OF CORN PLANTS WITH CROWN INFECTION WHEN GROWN IN SOIL INFESTED WITH *DIPLODIA ZEAE* ON STEAMED OATS AT DIFFERENT DATES AFTER EMERGENCE.

| Types of corn used | Time of soil infestation (days after emergence) | No. plants* with lesions |      | No. plants from which <i>Diplodia</i> was isolated |                 |                  |
|--------------------|-------------------------------------------------|--------------------------|------|----------------------------------------------------|-----------------|------------------|
|                    |                                                 | Crown                    | Stem | Crown                                              | First internode | Second internode |
| Sweet corn*        | 7                                               | 5                        | 4    | 3                                                  | 3               | 4                |
| " "                | 14                                              | 4                        | 3    | 5                                                  | 5               | 4                |
| " "                | 21                                              | 4                        | 2    | 5                                                  | 4               | 2                |
| " "                | 28                                              | 3                        | 1    | 3                                                  | 4               | 3                |
| " "                | 7 and 90                                        | 4                        | 3    | 5                                                  | 2               | 2                |
| " "                | 14 and 90                                       | 4                        | 2    | 5                                                  | 3               | 1                |
| " "                | 21 and 90                                       | 3                        | 3    | 5                                                  | 3               | 3                |
| " "                | 28 and 90                                       | 4                        | 2    | 5                                                  | 1               | 1                |
| " "                | Check                                           | 1                        | 0    | 0                                                  | 0               | 0                |
| Field corn**       | 7                                               | 7                        | 10   | 10                                                 | 10              | 8                |
| " "                | 35                                              | 6                        | 8    | 9                                                  | 9               | 6                |
| " "                | 70                                              | 3                        | 6    | 5                                                  | 5               | 3                |
| " "                | Check                                           | 0                        | 1    | 0                                                  | 0               | 0                |

\*Readings in each group based upon five plants.

\*\*Readings in each group based upon ten plants.

Although this artificially induced infection extended farther up the crown and stalk of these plants grown under greenhouse conditions than occurs in the field, the symptoms were typical of crown infection. Over 10 percent of the plants grown in infested soil matured from 1 to 3 weeks before any of the corresponding non-infected plants.

Since these experiments suggest that infected plant refuse might harbor the fungus, pieces of tissue from naturally-infected crown and stalk were added to steamed compost supporting 2-week-old corn seedlings grown from disease-free seed. The infected plant had matured in the field and remained outside until late in February. The soil in other containers was infected with *Diplodia zeae* growing either on agar or steamed whole oats.

The data secured at maturity showed (table 5) that infection resulted from naturally infected stalks as well as from pure cultures on steamed oats and corn meal agar. Infection was typical in both this experiment and subsequent tests where infected crowns were used as the source of inoculum.

It is possible that such crown infection derived from contaminated soil was induced directly by inoculum or infested soil which might have been splashed upon the stems or into the lower

TABLE 5. NUMBER OF CORN PLANTS WITH CROWN INFECTION DERIVED FROM STEAMED SOIL INFESTED BY DIFFERENT TYPES OF CULTURES.

| Type of inoculum used for soil infestation | No. plants* with lesions |      | No. plants from which <i>Diplodia</i> was isolated |                 |                  |
|--------------------------------------------|--------------------------|------|----------------------------------------------------|-----------------|------------------|
|                                            | Crown                    | Stem | Crown                                              | First internode | Second internode |
| Agar culture                               | 10                       | 6    | 9                                                  | 9               | 8                |
| Oat culture                                | 8                        | 5    | 9                                                  | 9               | 6                |
| Naturally infected corn                    | 7                        | 4    | 7                                                  | 7               | 6                |
| Check                                      | 1                        | 0    | 0                                                  | 0               | 0                |

\*Readings based on 10 plants in each group.

leaf sheaths. In order to prevent such aerial infection 40 plant cultures were sealed with paraffin-beeswax mixture immediately after the soil was infested with *Diplodia zeae* growing on steamed oats. The 3-week-old seedlings of field corn were protected from injury by a compact collar of cotton. At maturity, 80 percent of the plants had lesions on the crown. These extended above the paraffin seal and supported pycnidia of *Diplodia zeae*.

These data are in accord with the field observations of 1930 (table 1). In September there was less than 4 percent of typical crown infection, but the pathogene could be isolated from an additional 10 percent of the plants. About 25 days later, the fungus had progressed up to the crown and lower internodes sufficiently to give over 13 percent of typical infection. Some infection of the lower internodes by wind-borne spores undoubtedly occurs, but such infection can usually be distinguished by careful examination of the underground parts of the crown. Under greenhouse conditions the pathogene rarely extended more than two internodes downward from a direct wound inoculation into a growing stalk.

The progressive invasion of the crown from the soil might account, in part, for the unusually high percentage of infection on the lower nodes which Durrell (5) attributed to early loosening of the leaf-sheath and high relative humidity near the ground level. Durrell (5) attempted to inoculate plants growing under field conditions by placing oat cultures of *Diplodia zeae* on oats around the crown. His failure to confirm Smith and Hedges' (26) report of stalk invasion may be attributed to the fact that his readings were made too early in the season (Aug. 27).

*Diplodia zeae* is not systemic in the sense described by Smith and Hedges (26) and has never been observed to reach the ears from infested soil. Ear invasion, however, is not impossible for the conditions described in their report. The pathogene may progress four to six internodes above ground when the plant is grown under unfavorable conditions, such as exist in the greenhouse in mid-winter. Under better growing conditions, such as those existing in summer, the pathogene rarely progresses more than two internodes up the stalk of plants grown in ample containers.

#### INVASION AND PROGRESS OF INFECTION IN THE CROWN

Approximately 800 sweet corn seedlings, Golden Bantam variety, grown in 5-inch pots were divided into four equal groups. At intervals of 7, 15, 21, or 28 days after emergence of the seedlings the steamed compost in which they were growing was infested with *Diplodia zeae* growing on steamed oats. Approximately 20 plants were removed from each group at 7-day intervals after soil infestation and examined for lesions on the mesocotyls and crowns. Since plants in non-infested soil showed from 20 to 30 percent discolora-

TABLE 6. PERCENTAGE OF SWEET CORN PLANTS VAR. GOLDEN BANTAM, WITH NECROTIC LESIONS AFTER SOIL HAD BEEN INFESTED WITH *DIPLODIA ZEA* ON STEAMED OATS.

| Date of soil infestation | Percentages of mesocotyls with lesions<br>Dates examined* |           |            |            |            |           |           |            |
|--------------------------|-----------------------------------------------------------|-----------|------------|------------|------------|-----------|-----------|------------|
|                          | Nov.<br>28                                                | Dec.<br>5 | Dec.<br>12 | Dec.<br>19 | Dec.<br>26 | Jan.<br>2 | Jan.<br>9 | Jan.<br>16 |
| November 21              | 20                                                        | 60        | 45         | 40         | 40         | 80        | 80        | 70         |
| November 29              | --                                                        | 30        | 60         | 50         | 70         | 60        | 50        | 90         |
| December 5               | --                                                        | --        | 30         | 40         | 30         | 80        | 70        | 80         |
| December 12              | --                                                        | --        | --         | 29         | 25         | 75        | 33        | 66         |

\*Twenty plants examined at each date.

tion or lesions from mechanical injury or other outside agencies, only readings of more than 30 percent mesocotyl injury can be taken as indicative of infection by *Diplodia zea* invasion. The data presented in table 6 show that lesions appeared on the mesocotyl about 2 or 3 weeks after soil infestation.

*Diplodia zea* was never isolated from lesions on a mesocotyl prior to 10 days after infestation of the soil. The primary lesion usually occurred at the junction of the mesocotyl and seed but sometimes was distinctly above this point. In some instances the lesion was found on the crown where an adventitious root emerged. Approximately 30 percent mesocotyl infection was present 15 days after soil infestation. Thirty-five days later, however, only 30 percent of the mesocotyls were entirely decayed. At 75 days 25 percent infection of the bases of the crowns had occurred, and at 90 days this had increased to only 30 percent.

Although the progress of the fungus appeared to be checked after the crown was reached, 2 of the 10 plants remaining in the infested soil began to mature at 95 days, while all the plants in non-infested soil remained viable until 3 weeks later. At 115 days about 70 percent of the plants in infested soil showed infection above ground. In the comparatively brief period from 90 to 115 days after soil infestation, the infection increased from 30 percent on the crown to 70 percent one internode above ground.

Actually this apparent check and rapid subsequent development of the fungus is not as definite as it appears. Histological sections of the infected crowns of sweet corn plants growing under greenhouse conditions show that the lesion on the mesocotyl extends in advance of the mycelium as an internal deterioration of the parenchymatous cells at the base of the crown. At first the lesion is restricted to the region below the lower adventitious roots. Later, the pathogene extends through the outer layers of parenchymatous cells around the roots and up to the crown with very slight injury to the epidermis. The xylem vessels are discolored very frequently, even though the mycelium has never been identified in them. Discolored xylem vessels have been observed in the crowns of plants with infection restricted to the parenchymatous cells at the base of the



crown. A similar, but more extensive, discoloration of xylem in infected stalks and shanks has been illustrated by Durrell (5).

This progressive invasion of the crown from previous mesocotyl infection has been ignored for the most part. Raleigh (22) has suggested that prematurity in sweet corn may be due to such invasion from infected seed. Holbert, Hoppe and Smith (8) have recently described a pronounced spread of *Diplodia zeae* in artificially inoculated stalks as the plants approach maturity. They would associate such invasion with a decrease in the food reserves of the stalks.

#### EFFECT OF SEED TREATMENT ON LATE CROWN INFECTION

Samples of *Diplodia*-infected and nearly-disease-free seed were treated with an organic mercury dust containing 4.2 percent of white precipitate of mercury (mostly as  $\text{NH}_2\text{HgCl}$ ) in a talc filler and planted alongside non-treated seed in 1930 and 1931. Corn had been planted in the field the preceding years. Following maturity each plant was pulled and examined for crown infection. The percentages of infection given in table 7 are based solely upon those plants supporting pycnidia of *Diplodia zeae* on the crown and lowest internode.

Although there was more infection in three of the four groups where there was a larger number of surviving plants, this could not be correlated with density of stand. No significant differences could be found in the percentage of crown infection of corn grown from treated and non-treated disease-free and *Diplodia*-infected seed planted at the rate of two, three, four and five kernels per hill, or with the stand thinned to a corresponding number of plants per hill. Five replications of 25 hills each were observed.

Although there was much more infection in 1931 than in 1930 (table 7), the relative amount of infection on plants from disease-free and *Diplodia*-infected seed under different treatments was comparable. In both years the infected seed gave a higher percentage of crown infection than did disease-free seed. Furthermore, the data indicate that treatment of disease-free seed decreased the percentage of crown infection, while treatment of infected seed increased infection. Since the statistical significance of these differences was questionable, the following experiments on the effect of seed treatment under controlled conditions were conducted.

Treatment of disease-free seed might decrease the percentage of

TABLE 7. THE EFFECT OF SEED TREATMENT UPON THE AMOUNT OF CROWN INFECTION OF PLANTS GROWN FROM INFECTED AND NON-INFECTED SEED.

| Seed treatment | Character of seed         | Oct. 20, 1930 |                    | Nov. 1, 1931 |                    |
|----------------|---------------------------|---------------|--------------------|--------------|--------------------|
|                |                           | No. plants    | Infection, percent | No. plants   | Infection, percent |
| Not treated    | Disease-free              | 217           | 12.4               | 166          | 58.4               |
| Mercury dust   | "                         | 225           | 9.8                | 151          | 47.7               |
| Not treated    | <i>Diplodia</i> -infected | 160           | 16.9               | 107          | 71.0               |
| Mercury dust   | "                         | 172           | 18.6               | 116          | 75.5               |

crown infection by delaying invasion of the mesocotyl from infested soil. As shown above a delay at the time of infestation reduces the percentage of infection. To test this possibility, treated and untreated disease-free seed were planted in a flat heavily infested with *Diplodia zeae* growing on steamed whole oats. Fourteen days later 11 percent of the 50 plants from the treated seed and 75 percent of those from untreated seed had lesions on the mesocotyl. After 23 days these percentages had increased to 14 and 91 percent, respectively. Under these conditions a thorough coating of organic mercury dust on the seed retarded infection of the seedlings from the infested soil. An increase in yield attributable to reduction in crown infection derived from infested soil remains to be demonstrated. It may account in part, however, for the beneficial effects reported (24) (23) (9) from treating disease-free seed.

If treatment of infected seed increases the percentage of crown infection, as indicated in table 7, the only apparent explanation is that treatment inhibits the fungus without killing it. Under these conditions, many plants not killed as seedlings might be invaded later. This was shown to be the case in an experiment in which 400 *Diplodia*-infected seeds were divided into two lots, one of which was soaked in a heavy suspension of organic mercury dust (4.2 percent white precipitate of mercury in talc) and the other in tap water for 15 hours. The seeds were germinated on a moist blotter at 16°C. After 7 days 80 percent of the treated seeds had germinated and none of the seedlings had lesions on the mesocotyl. In the untreated lot 60 percent germinated and 44 percent were infected. After 14 days the treated seedlings were still free from lesions, while the untreated seedlings were 94 percent infected. After 3 weeks, when all of the untreated lot except one had died, small lesions had appeared on 80 percent of the seedlings in the treated lot. *Diplodia zeae* was isolated from 50 percent of these lesions, only one of which was fatal at 4 weeks after germination.

The prolonged treatment in an organic mercurial soak effectively protected the plant through the seedling stage even though the pathogene was not destroyed. Apparently the organic mercury treatment merely inhibited the mycelium which was protected from destruction by the seed tip cap. The organic mercury dusts have been brought to a high state of efficiency since Reddy began investigating chlorophol and later cooperated with Holbert in conducting field tests, the results of which were published in 1924 (24). Although the seedling-blight stage of *Diplodia zeae* on the corn plants may be largely controlled, the prevention of crown infection offers an additional field of effort in the perfection of seed disinfectants.

The various data presented above are offered as evidence that the very prevalent infection on the crown and lower nodes of corn stalks is, to a large measure, the result of underground invasion rather than

of aerial infection by wind-borne spores. Such infection may be derived either from infected seed or soil infested by infected plant refuse. In either case the mesocotyl is usually invaded at the wounds created by the emergence of seminal roots and gradually decayed. The seedling does not die since the mesocotyl is not girdled before the adventitious roots are established. Seed treatment may affect crown infection by (1) preventing the death of seedlings grown from *Diplodia*-infected seed without actually killing the pathogene and thereby eliminating all possibility of delayed infection and (2) by retarding the parasitic invasion of the mesocotyl from infested soil. With these observations on the nature of crown infection in mind, the next section will be devoted to the factors affecting the pathogenicity of *Diplodia zeae* in the soil and the effect of crown infection on the plant's development.

#### FACTORS INFLUENCING CROWN INVASION FROM INFESTED SOIL AND ITS EFFECT UPON THE HOST

There is little definite information on the factors influencing growth of *Diplodia zeae* in the soil. Durrell (5) and Van der Bijl (1) observed that steamed compost could be used as a medium for pure cultures. Burrill and Barrett (3) have shown that viable spores can be obtained from pycnidia in host tissues which have remained in the soil for several seasons. The ability of the fungus to exist as a free-living entity in the soil and invade growing plants, however, has never been demonstrated experimentally. Smith and Hedges (26) described a stalk invasion from artificially infested soil, but their data have received little consideration since their description of systemic invasion was questioned by Durrell (5).

The following studies were initiated to determine whether the pathogene could exist as a soil saprophyte, whether it could be recovered from field soil, and whether soil texture and moisture had any influence upon its development in the soil. Such factors have been associated with parasitic activity and the effect of crown infection upon the development and water economy of the host.

#### EFFECT OF SOIL MOISTURE UPON THE SAPROPHYTIC EXISTENCE OF *DIPLODIA ZEA* IN THE SOIL

In order to interpret the various aspects of crown invasion from infested soil it was necessary to determine the nature and extent of the saprophytic existence of *Diplodia zeae* in the soil. The tests reported below were conducted to determine if the pathogene could live in a soil devoid of host tissue and if the condition of the soil had any effect upon its development.

Steamed moist soil in petri dishes 12 cm. in diameter was infested by inserting *Diplodia zeae* on a quarter-inch cube of corn meal agar. Small sections of infected roots, an oat kernel from a culture on steamed oats, or infested soil served equally well when used as inoculum. The fungus grew throughout the soil and, after about 17



TABLE 8. SQUARE INCHES OF STEAMED GREENHOUSE COMPOST COVERED BY *DIPLODIA ZEA*E AT DIFFERENT SOIL MOISTURE CONTENTS.

| Initial percent soil moisture | Time of observation |           |          |
|-------------------------------|---------------------|-----------|----------|
|                               | Four days           | Five days | Six days |
| 15.3                          | 0.53                | 0.98      | 0.98     |
| 30.0                          | 3.28                | 5.43      | 7.93     |
| 46.3                          | 3.50                | 8.10      | 15.60    |
| 68.4                          | 0.42                | 2.20      | 3.70     |
| 90.1                          | 0.00                | 0.00      | 0.34     |

days, produced pycnidia at the junction of the soil and the side of the petri dish. The progress of the mycelium along the bottom of the petri dish could be determined from day to day by holding the dish at an oblique angle to direct light. The fungus grew abundantly in peat soil and well in compost and field soil, but not in sand. Dilution of compost with sand decreased the growth rate.

The lateral spread of *Diplodia zeae* in soil cultures was retarded by contamination of the culture with *Rhizopus* and, to a lesser extent, by *Penicillium*. Fair growth, however, was made in compost with the natural flora undisturbed by steaming. The fungus was recovered at least 4 centimeters from the point of soil infestation in 2 out of 10 petri dishes filled with unsteamed compost. Furthermore, as reported below, the fungus was recovered from field soil planted with corn the preceding season by growing corn plants from disease-free seed in it. These facts would indicate that the fungus has at least a limited existence as a free-living entity in the soil.

Six petri dishes were filled with compost adjusted to different soil moisture contents and steamed for 30 minutes at 15 pounds pressure. After steaming the moisture contents were 15.3, 30.0, 46.3, 68.4 and 90.1 percent of the water-holding capacity (43 percent). The soil in each dish was infested with a small cube of corn meal agar supporting *Diplodia zeae* and incubated at 28°C. The area covered by the mycelium was marked with a wax pencil, traced on paper and measured by a planimeter. As shown in table 8, the optimum soil moisture content for lateral spread appeared to be about 40 percent of the water holding capacity. At 15.3 percent it was observed that growth was restricted to the area in contact with the agar cube. No growth occurred in the 90 percent soil until the moisture dropped to below 70 percent on the sixth day. The moisture content in the third dish dropped from 46.3 percent to 36 percent during the incubation period.

The amount of soil moisture controlled the relative position of the mycelium in the soil. At the lowest content all growth was along the bottom of the dish, while at the higher contents it was sharply limited to the surface.

#### EFFECT OF SOIL MOISTURE UPON CROWN INVASION FROM INFESTED SOIL

An attempt was made to correlate the prevalence of infection with

soil moisture in order to explain the pronounced differences in amount of crown infection in different years (tables 1 and 7). Three experiments were conducted, as outlined below, the plants being grown in sealed containers outside the greenhouse. Data on the extent of crown invasion at maturity and reduction in total dry weight of plants grown at different soil moistures are presented. These data have been presented separately since it was found that the growing conditions for the plant controlled the severity of injury rather than the extent of invasion. Since the soil moisture had to be readjusted periodically the amount of water used by each plant was recorded and used to compute the transpiration ratios given in the second chapter below.

#### METHODS

In all of the experiments on controlled soil moisture, the water-holding capacity of the soil was determined by soaking a weighed quantity of soil of known moisture content for 2 hours in a 1-gallon glazed jar with the drain outlets protected by several folds of cheesecloth. The jars were covered with a glass plate, sealed with vaseline and allowed to drain for several days until the cheesecloth began to dry. The containers were reweighed and the percentage moisture computed. The wilting coefficient of the soil was determined after holding 4-week-old plants in sealed pots until they wilted and would not recover in a saturated atmosphere.

The containers were filled with a given quantity of bin soil of known moisture content and infested with oat cultures. The seed was planted and the covers adjusted. After emergence the seedlings were protected from injury by insertion of a loose cotton plug between the stalk and metal cover and were sealed into the container by a closely wrapped oilcloth collar. Correction for the weight of the plant was made twice during the summer by comparing it with a field plant of similar size.

The tops of the plants were cut off at maturity and the core of soil worked out of the container upon a screen. The soil was washed from the roots and those roots which had broken off were recovered from the screen. Readings were taken on infection, and the plant parts were dried to a constant weight at 103°C.

#### PRELIMINARY EXPERIMENTS

Field corn was grown in 4-gallon jars capable of holding 13 kilograms of oven-dry (calculated) soil which was adjusted to either 30, 45, 90, or 90 which was later dropped to 45 percent of the water-holding capacity of the soil. When the plants at optimum soil moisture had obtained a height of over 6 feet, they were using over 25 percent of the total water supply in the jar each day, making an accurate control of the soil moisture content impracticable. Under these conditions the different soil moisture levels overlapped and



Fig. 4. Development of field corn grown in *Diplodia*-infested and non-infested soil held at 30 percent of the water-holding capacity. The three plants in the back row were grown in non-infested soil, the two in the front in *Diplodia*-infested soil. The lesions on the infected plants did not extend far up the crown, but the top growth was reduced very severely and the plants died 2 weeks before the checks. For differences in growth of infected and non-infested plants at 70 and 90 percent soil moisture see figs. 5 and 6.

probably represented a range from minimum to optimum rather than from minimum to maximum.

The compost in six jars at each soil moisture content was infested with *Diplodia zeae* growing on steamed whole oats, and a comparable, non-infested series was retained. Soil from a field planted in corn the preceding year was used in a third series with five jars held at each soil moisture content. The field soil had overwintered in the field. All pots were planted in May with disease-free seed of open-pollinated field corn. When the plants were examined for infection in September, all of the plants grown in artificially infested soil had lesions on the mesocotyl and crown. At the lower moisture content, however, the crowns were only lightly discolored at the very base, whereas the crowns of the plants at the higher soil moisture contents were invaded above the ground line and extensively discolored.

Infection from the naturally infested soil was so scattering that the effect of soil moisture could not be determined. Only 3 of the 20 plants had lesions typical of *Diplodia zeae* infection, and pycnidia were abundant on only one of the plants. Since none of the plants grown in compost from the same lot of seed was infected, this crown infection presumably was derived from the field soil.

These observations were substantiated by a second experiment using sweet corn, Golden Bantam variety, grown in 1-gallon glazed jars containing steamed compost adjusted to 30, 45, 70, 90, or 90 later dropped to 45 percent of the water-holding capacity of the soil. At maturity these plants were found to have been invaded most severely at the higher soil moisture contents even though growth was more reduced at the lowest level. Since the moisture content of the soil could not be rigidly controlled in the small containers, the detailed data are not presented. In order to control the soil moisture more accurately it was deemed necessary to repeat these experiments using the more suitable equipment described below.

#### EXPERIMENT OF 1932

Greenhouse compost was weighed into galvanized iron cans 26 by 15.7 inches in size which were capable of holding 165 pounds of oven-dry (calculated) soil. The covers were sealed with mechanic's tape and the transpired water was replaced by pouring through a  $\frac{3}{4}$ -inch hole into an inverted 6-inch pot. The equipment was essen-



Fig. 5. Development of field corn grown in *Diplodia*-infested and non-infested soil held at 70 percent of the water-holding capacity of the soil. The three plants in the back row were grown in non-infested soil, the three in the front in *Diplodia*-infested soil. Although the plants grown in *Diplodia*-infested soil had lesions extending into the crown, their top growth was not appreciably retarded and they matured about the same time as their checks. This soil moisture content appeared to be the optimum for growth both years that the experiments were conducted. For the difference in growth of infected and non-infected plants at 30 and 90 percent soil moisture see figs. 4 and 6.



TABLE 9. DEGREE OF INFECTION ON CORN PLANTS GROWN AT DIFFERENT SOIL MOISTURE CONTENTS IN NON-INFESTED SOIL AND SOIL INFESTED WITH *DIPLODIA ZEAE*, 1932.

| Condition of soil |                         | Condition of plant parts |                         |                        |                  |
|-------------------|-------------------------|--------------------------|-------------------------|------------------------|------------------|
| Infestation       | Soil moisture contents* | No. plants maturing      | No. crowns with lesions | No. stems with lesions | Root description |
| Non-infested      | 90                      | 5                        | 0                       | 0                      | 5 normal         |
| Non-infested      | 70                      | 5                        | 0                       | 0                      | 5 normal         |
| Non-infested      | 55                      | 4†                       | 0                       | 0                      | 4 normal         |
| Non-infested      | 30                      | 5                        | 0                       | 0                      | 5 dried up**     |
| Diplodia-infested | 90                      | 5                        | 5                       | 4                      | Brown rot        |
| Diplodia-infested | 70                      | 5                        | 5                       | 3                      | Light brown      |
| Diplodia-infested | 55                      | 5                        | 5                       | 2                      | Discolored       |
| Diplodia-infested | 30                      | 5                        | 3                       | 0                      | Normal           |

\*Percentage of waterholding capacity.

\*\*Plants were replanted (see text).

†The other plant in this group was infected with *Diplodia zeae*, apparently from soil contamination.

tially that described by Briggs and Shantz (2). The soil moisture content was adjusted to either 30, 55, 70, or 90 percent of the water-holding capacity, which was 42 percent of the dry weight. The wilting coefficient was 10.8 percent of the dry weight.

The soil in 5 of the 10 cans at each soil moisture content was infested with *Diplodia zeae* growing on whole oats. The plants grown from open-pollinated seed corn (Pfister's Krug) under outside conditions from May until September in 1932 made a normal growth comparable to that reported by Miller (19) and Kiesselbach (13). The soil moisture control was very effective, never varying more than 5 percent. Examination of the soil when the experiment was discontinued in September showed a very uniform distribution of the roots and moisture except for the upper inch, which was slightly drier than the remainder. In the series approaching saturation, there was some free water in the lower half of the soil. In order to prevent aggregation of the roots near the water source, the plants grown at 30 percent soil moisture were watered heavily every second or third week and left until the available water was used. The two groups held at 30 percent soil moisture (table 10) are not comparable since some of the sweet corn plants in the non-infested soil died from excessive drying and were replanted.

As in the preceding experiment, the infection was most apparent at higher soil moisture contents. The data presented in table 9 show that the crowns of all plants grown in infested soil were invaded, with the exception of two plants at 30 percent soil moisture. However, the mesocotyls were killed in all the plants and the bases of the crowns were infected.

#### EXPERIMENT OF 1933

The 1932 experiment was repeated in 1933 using a line of field corn which had been selfed for eight generations (OSF of the Genetics Section of Iowa State College). The plants made less growth than those in 1932 and were subject to outside injury. Al-



Fig. 6. Development of field corn grown in *Diplodia*-infested and non-infested soil held at 90 percent of the water-holding capacity of the soil. The three plants in the back row were grown in non-infested soil, the two in the front row in infested soil. The plants grown in *Diplodia*-infested soil were severely infected having lesions extending through the crown and into the lower internodes. The infected plants matured early and never developed ears as did the check. For the differences in growth between infected and non-infested plants at 30 and 70 percent soil moisture see figs. 4 and 5.

though some plants died from sunscald in the seedling stage, there were at least four plants available in each group for a final reading, except in the 90 percent series which had three. The data secured approximated the 1932 data.

The severity of crown invasion does not appear to be correlated with soil moisture conditions favoring fungous growth in the soil. As pointed out before, the fungus grew best at a soil moisture content of about 40 percent. Crown invasion, however, was not very extensive at 55 percent. At 90 percent, where crown invasion was pronounced, the fungus made poor growth in the soil. It has not been determined whether the soil moisture content affects the susceptibility of the growing host to invasion, or merely promotes lateral spread of the fungus within the host tissue as the tissue dies.

#### REDUCTION IN DEVELOPMENT OF THE PLANT BY CROWN INFECTION

In all the experiments reported in the preceding pages, the plants were dried to a constant weight at 103°C. The dry weights of the field corn in the preliminary experiments were not taken because the oven burned some of the plants. However, the sweet corn, Golden Bantam variety, grown in 1-gallon jars as a preliminary experi-

TABLE 10. DRY WEIGHTS IN GRAMS OF SWEET CORN PLANTS GROWN AT DIFFERENT SOIL MOISTURE CONTENTS IN NON-INFESTED AND DIPLODIA-INFESTED SOIL.

| Soil moisture content | Dry weight of tops |                |                     | Dry weight of roots |                |                     | Dry weight complete plant |                |                     |
|-----------------------|--------------------|----------------|---------------------|---------------------|----------------|---------------------|---------------------------|----------------|---------------------|
|                       | Non-infested soil  | In-fested soil | Coefficient* growth | Non-infested soil   | In-fested soil | Coefficient* growth | Non-infested soil         | In-fested soil | Coefficient* growth |
| 30                    | 40.9               | 37.7           | 0.922               | 19.2                | 13.9           | 0.724               | 60.1                      | 51.6           | 0.858               |
| 45                    | 119.4              | 120.2          | 1.007               | 40.9                | 24.1           | 0.589               | 160.3                     | 144.3          | 0.900               |
| 70                    | 178.8              | 166.8          | 0.933               | 49.4                | 38.1           | 0.771               | 228.2                     | 204.9          | 0.898               |
| 90                    | 191.4              | 192.3          | 1.005               | 46.5                | 38.0           | 0.871               | 237.9                     | 230.3          | 0.968               |
| 90-45                 | 141.3              | 133.0          | 0.941               | 39.7                | 26.8           | 0.675               | 181.0                     | 159.8          | 0.883               |

\*Weight of plants in infested soil divided by weight of corresponding plants in non-infested soil.

ment, was weighed. Some of the roots may have been lost in washing, particularly when they were decayed, but the dry weights presented in table 10 are representative of the comparative development of the plants.

The plants grown in Diplodia-infested soil had a total dry weight 91 percent of that of corresponding plants grown in non-infested soil, most of this reduction being in root development (fig. 7).

The more refined experiments of 1932 and 1933 (in which Pfister's Krug open-pollinated and a selfed line, OSF, of dent corn seed were used, respectively) extended these observations to include plants grown at soil moisture contents definitely above the optimum for plant development. The plants were grown, as stated above, in galvanized iron cans in which the soil moisture could be held within 5 percent of the desired level. Therefore the average weights of the five oven-dried plants in each group, which are presented in table 11, are of plants grown at soil moisture contents rigidly controlled at the stated levels.

TABLE 11. MEAN DRY WEIGHT IN GRAMS OF FIVE CORN PLANTS GROWN AT DIFFERENT SOIL MOISTURE CONTENTS IN NON-INFESTED AND DIPLODIA-INFESTED SOIL IN 1932 AND 1933.

| Condition of soil     | Soil water percent | 1932                 |        |        |                       | 1933                 |       |       |                       |
|-----------------------|--------------------|----------------------|--------|--------|-----------------------|----------------------|-------|-------|-----------------------|
|                       |                    | Dry weight per plant |        |        | Coefficient of growth | Dry weight per plant |       |       | Coefficient of growth |
|                       |                    | Tops                 | Roots  | Total  |                       | Tops                 | Roots | Total |                       |
| Non-infested          | 90                 | 186.92               | 193.36 | 384.28 | 1.00                  | 51.4                 | 14.5  | 65.9  | 1.00                  |
| Non-infested          | 70                 | 297.74               | 123.90 | 421.64 | 1.00                  | 77.4                 | 20.8  | 98.2  | 1.00                  |
| Non-infested          | 55                 | 384.85               | 190.65 | 575.40 | 1.00                  | 68.8                 | 17.7  | 86.5  | 1.00                  |
| Non-infested          | 30*                | 62.32                | 36.44  | 98.76  | ----                  | 26.5                 | 7.0   | 33.5  | 1.00                  |
| Non-infested average  |                    | 283.05               | 169.18 | 452.23 | 1.00                  | 58.0                 | 15.51 | 73.53 | 1.00                  |
| Diplodia-infested     | 90                 | 105.80               | 61.42  | 167.22 | .44                   | 33.9                 | 8.7   | 43.6  | .66                   |
| Diplodia-infested     | 70                 | 362.28               | 61.22  | 423.50 | 1.00                  | 80.4                 | 18.1  | 108.6 | 1.10                  |
| Diplodia-infested     | 55                 | 323.22               | 70.05  | 393.27 | .68                   | 50.7                 | 17.1  | 67.8  | .78                   |
| Diplodia-infested     | 30                 | 204.26               | 92.78  | 297.02 | ----                  | 17.8                 | 4.1   | 21.9  | .65                   |
| Diplodia-infested av. |                    | 267.48               | 64.59  | 332.07 | .73                   | 49.5                 | 12.48 | 62.0  | .84                   |

\*This series died out and was replanted, so it is not comparable to the corresponding inoculated group.

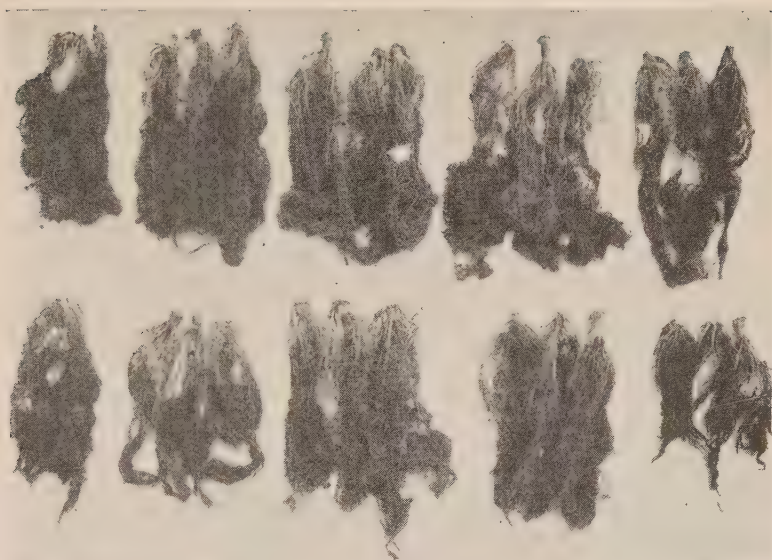


Fig. 7. Condition of the roots of sweet corn plants, Golden Bantam variety, grown in *Diplodia*-infested soil (bottom row, "F" to "J") and plants grown in non-infested compost (upper row, "A" to "E") at five soil moisture contents. "A" and "F" were held at 30 percent of the water-holding capacity, "B" and "G" at 45, "C" and "H" at 70, "D" and "I" at 90, and "E" and "J" at 90 for 46 days then dropped to 45 percent for the remaining 49 days before harvesting. Apparently *Diplodia zeae* is most destructive at moisture contents either below or above optimum. The sudden shift from 90 to 45 percent in "E" and "J" stopped growth, and the roots were decayed considerably by *Diplodia zeae* before the remainder of the plants were ready for harvest.

In 1932 the dry weight of plants grown in infested soil was reduced to 44 percent of the corresponding grown in non-infested series at a high soil moisture, and to 68 percent at a low soil moisture. Under optimum growing conditions (70 percent) there was no appreciable reduction in total dry weight. Although the dry weight of the roots was more reduced than that of the tops, the latter was only 57 percent of normal at 90 percent soil moisture. The total dry weight of all four groups of plants growing in infested soil was only 73 percent of the non-infested checks. The data secured in 1933 confirmed those of 1932. There was no developmental failure of plants grown in infested soils at optimum conditions (fig. 5). However, those grown at low (fig. 4) or high (fig. 6) soil moisture contents produced only 65 and 66 percent as much dry matter as plants grown in non-infested soil. The dry matter produced by all plants grown in infested soil was 84.3 percent of the non-infested checks.

A further test of the effect of crown infection on corn was to determine if there is a difference in yield between infected and non-infected plants. A field at Kanawha, Iowa, in which corn had not been planted for several years, was planted at the rate of three plants



per hill in 1934. At maturity the individual ears from 2,000 plants in 28 plots were weighed. In 23 of the 28 replications of plants, the average weight per ear from infected plants was less than that of non-infected plants. The average of all groups showed 2.2 percent less ear corn produced on the plants suffering crown infection.

The amount of crown infection was less than 5 percent in this field. The lesions were not pronounced and did not extend very far up the stalk. This light infection may be attributed to the limited amount of inoculum in the soil and to the very favorable growing season. It is striking that under conditions least favorable to crown infection the reduction in yield amounted to 2.2 percent. The usual reduction in yield on land used more frequently for corn probably falls between this figure and the 10 percent reduction in yield from plants with "severely infected lower nodes" reported by Durrell (5).

This decrease in yield of ear corn and production of total dry matter is difficult to explain. The fungus invades the entire crown so late in the developmental history of the plant that it should not cause such pronounced injury. When infection is derived from soil, the mesocotyl is not destroyed sufficiently early to reduce seedling vigor and thereby later affect the yield as has been shown for seed-borne infection by Holbert *et al* (6). It is possible that the loss of the primary root system during the period of active growth is more important than is generally considered.

Although the mesocotyl and primary radicle are usually considered as a "temporary root system," the loss of these parts is not a natural phenomenon in healthy plants. They die because of the invasion of the mesocotyl by soil-inhabiting parasites such as *Diplodia zae*. When plants are grown in steamed compost safeguarded against contamination, the mesocotyl and radicle remain clear white, develop lateral branches and grow to a length of 20 or more inches. These roots function until maturity as can be shown by growing the plant entirely upon them in the absence of adventitious roots.

A corn plant grew 61 inches high and supported an ear 5 inches long when grown only on the primary root system in the greenhouse from February until May. The primary root system was grown in steamed compost in an 8-inch pot, while the crown developed in another superimposed 8-inch pot filled with dry sand. All adventitious roots were sheared off as soon as they appeared, leaving the undisturbed primary roots to support the plant. The primary radicle grew at least 6 feet in length and developed an intricate fibrous system. Apparently the primary root system is capable of assisting in the support of the plant, but its relative importance in the presence of the adventitious roots has not been evaluated.

## EFFECT OF CROWN INFECTION ON THE TRANSPIRATION RATIO

The amount of water used by the plants grown under the controlled soil moisture conditions reported above was recorded each day. The transpiration ratio (water requirement) of each group was calculated from these records and the dry weights of the plants. The 25 sweet corn plants (table 10) grown in infested soil at the four different soil moisture contents had an average transpiration ratio of 277 as compared to 245 for the plants grown in non-infested soil. Corresponding to the range of moisture from optimum to minimum for growth, there was an increase in the transpiration ratios of 5, 13, 19 and 25 percent over the non-infected plants at the several soil moisture levels.

The data presented in table 12 for the field corn plants grown in larger containers in 1932 and 1933 give the difference in transpiration ratio of plants at moisture contents above optimum as well as below it. The transpiration ratio of all infected plants was more than that of non-infected plants both years, the average increase being 34 and 9 percent, respectively, for the 2 years. There was very little increase in transpiration ratio of infected plants at optimum soil moisture in either year, but as the soil moisture was varied either way from this point the increase in ratio was accentuated.

The transpiration ratio of non-infected plants observed in these experiments agrees very well with ratios reported by Miller (19). He found that plants with dry top weights of 250 to 450 grams have transpiration ratios of 231 to 475 in different years. The plants grown from open-pollinated seed in 1932 produced over 300 grams of dry matter at optimum soil moisture and had transpiration ratios of 219 to 317 under different soil moisture conditions.

## REACTION OF SELFED LINES TO LATE CROWN INFECTION

All of the plants of 20 selfed lines in a crossing block were pulled in October 1932 and examined for late crown infection. With the exception of some of the extracted (Extr.) lines, they had been selfed by the Genetics Department at Iowa State College for 8 to 9 years. Crown infection of the various lines ranged from 20 to 93 percent, as shown in table 13. Most of the lines showed a strikingly uniform reaction. For example, rows representing five different ears of "Bls" showed 85.7, 85.7, 83.3, 75.0 and 73.0 percent infection; "Pr" 14.3, 21.4, 21.4, 31.3; and "OSF" 100, 94, 88, 93 and 93 percent infection.

Certain lines, however, showed a distinct segregation, the plants from one ear being lightly infected while the others were severely infected. Such is the case in "Blx" which showed infection of 50.0,

TABLE 12. WATER CONSUMPTION AND TRANSPIRATION RATIO OF INFESTED AND NON-INFESTED CORN PLANTS GROWN AT DIFFERENT SOIL MOISTURE CONTENTS IN 1932 AND 1933.

| Soil conditions          |                          | Dent corn grown in 1932   |                       |                   |                                         |              |                           | Selfed line grown in 1933 |                   |                                         |              |             |  |
|--------------------------|--------------------------|---------------------------|-----------------------|-------------------|-----------------------------------------|--------------|---------------------------|---------------------------|-------------------|-----------------------------------------|--------------|-------------|--|
| Moisture content percent | Infestation with D. zeae | Amount of water used gms. | Average dry weight of |                   | Transpiration ratio based on dry weight |              | Amount of water used gms. | Average dry weight of     |                   | Transpiration ratio based on dry weight |              | Coefficient |  |
|                          |                          |                           | Tops gms.             | Entire plant gms. | Tops                                    | Entire plant |                           | Tops gms.                 | Entire plant gms. | Tops                                    | Entire plant |             |  |
| 90                       | none                     | 44,740                    | 186.9                 | 384.3             | 239.4                                   | 116.4        | 20,647                    | 51.4                      | 65.9              | 402                                     | 313          | 1.00        |  |
| 70                       | "                        | 67,800                    | 297.7                 | 421.6             | 227.8                                   | 160.8        | 25,919                    | 77.4                      | 98.2              | 335                                     | 264          | 1.00        |  |
| 55                       | "                        | 85,900                    | 384.9                 | 575.4             | 223.2                                   | 149.3        | 23,012                    | 68.9                      | 86.5              | 334                                     | 266          | 1.00        |  |
| 30                       | "                        | 6,760                     | 62.3                  | 98.8              | -----                                   | -----        | 12,222                    | 26.5                      | 33.5              | 462                                     | 365          | 1.00        |  |
| Average                  |                          | 64,736                    | 283.05                | 452.23            | 228.7                                   | 143.15       | 20,999                    | 58.0                      | 73.5              | 362                                     | 286          | 1.22        |  |
| 90                       | infested                 | 33,640                    | 105.8                 | 167.2             | 317.9                                   | 201.2        | 16,700                    | 33.9                      | 43.6              | 492                                     | 383          | 1.00        |  |
| 70                       | "                        | 79,440                    | 362.3                 | 432.5             | 219.3                                   | 187.6        | 30,095                    | 90.4                      | 108.6             | 333                                     | 277          | 1.05        |  |
| 55                       | "                        | 75,580                    | 323.2                 | 393.3             | 234.0                                   | 192.2        | 20,498                    | 50.7                      | 67.8              | 404                                     | 302          | 1.14        |  |
| 30                       | "                        | 43,200                    | 204.3                 | 297.0             | -----                                   | -----        | 9,200                     | 17.8                      | 21.9              | 517                                     | 420          | 1.15        |  |
| Average                  |                          | 63,675                    | 260.7                 | 332.1             | 244.2                                   | 191.75       | 19,344                    | 49.5                      | 62.0              | 391                                     | 312          | 1.09        |  |

TABLE 13. NUMBER AND PERCENTAGE OF INFECTED PLANTS OF VARIOUS SELFED LINES OF CORN IN OCTOBER 1932.

| Line examined | No. plants | No. plants<br>with <i>Diplodia</i> | Percentage of<br>crown infection |
|---------------|------------|------------------------------------|----------------------------------|
| Extr La       | 66         | 34                                 | 51.5                             |
| Extr Ldg      | 67         | 42                                 | 62.7                             |
| Extr Bls      | 70         | 41                                 | 58.6                             |
| Extr Blx      | 70         | 36                                 | 51.4                             |
| Extr Osf      | 68         | 42                                 | 61.8                             |
| Extr Lo       | 70         | 24                                 | 34.3                             |
| Extr Cl       | 73         | 52                                 | 71.2                             |
| La            | 61         | 33                                 | 54.1                             |
| Ldg           | 70         | 14                                 | 20.0                             |
| Bls           | 69         | 56                                 | 81.2                             |
| Blx           | 75         | 26                                 | 34.7                             |
| Lo            | 43         | 29                                 | 67.4                             |
| Pr            | 58         | 13                                 | 22.4                             |
| Osf           | 75         | 70                                 | 93.3                             |
| Ose           | 77         | 49                                 | 63.6                             |
| Cl            | 65         | 38                                 | 58.5                             |
| Mc            | 66         | 14                                 | 21.2                             |
| Lat           | 36         | 27                                 | 75.0                             |
| Las           | 39         | 18                                 | 46.2                             |
| Se            | 80         | 22                                 | 28.5                             |

50.0, 7.7 and 56 percent. The various lines seemed to be very uniform in stalk and root characters other than crown infection.

The percentage infection based on all four or five rows of a given line is presented in table 13. There are obvious differences in reaction.

### DISCUSSION

Crown infection, as such, has not been previously described. The early stages have been adequately discussed by those investigators interested in seedling blight. Manns and Adams (16) have illustrated the dormant mycelium under the seed tip cap, and Raleigh (22) has discussed its progress to the wounds created by the emergence of seminal roots at the base of the mesocotyl. He reported that infection was more severe when seedlings were grown in moist soil at 15° to 19°C. than at 20° to 24°C. These results have been substantiated by field observations reported by Raleigh (22), Holbert, *et al.* (6), Holbert, Koehler and Dungan (10) and Koehler (11).

Under more favorable growing conditions, the plant establishes roots before girdling of the mesocotyl by the parasite deprives the seedling of its food and water supply [Raleigh (22), Koehler and Holbert (15), Clayton (4) and Holbert *et al.* (7)]. The natural resistance of the adventitious roots [Durrell (5), Koehler and Holbert (15), Clayton (4)] prevents the destruction of the plant after it is established. Although there have been no critical observations on the subsequent development of the fungus on the mesocotyls of these infected plants which do not die as seedlings, the general opinion apparently has been that the fungus ceases activity. Raleigh (22) is the only investigator to suggest that the fungus might spread to aboveground parts. He observed *Diplodia zeae* on the lower nodes of sweet corn plants prematurely dead in August, 1927.



The invasion of the plant from infested soil has been a disputed question. Smith and Hedges (26) reported a general systemic invasion of plants grown over *Diplodia zeae* inoculum, but this interpretation was later discredited as a field phenomenon by Durrell (5), Clayton (4) and Van der Bijl (1). Durrell (5) secured a moderate infection of the basal part of the crown from heavy infestation of the soil in a flat, but under field conditions similarly inoculated plants were scarcely injured. Van der Bijl (1) induced a fatal infection by needle inoculation of the underground parts during a season of light rainfall but could not repeat his results during a normal growing season.

From the experimental data presented it seems that *Diplodia zeae* is very consistent in continuing its parasitic development into the growing plant either from infected seed or infested soil. The invasion, however, does not become systemic and does not extend far beyond the crown unless growing conditions are exceptionally poor. Since the fungus does continue to parasitize the plant in its more mature stages, it is obvious that seed treatments should be applied to eradicate the fungus rather than merely to inhibit it for a few weeks. Field and laboratory data indicate that at least one of the common organic mercury dusts fails to destroy the dormant mycelium even though it very effectively inhibited it sufficiently to prevent seedling blight. Infected seed treated with such dusts produces plants which do not die as seedlings but later suffer crown infection. However, the ability of the dust to inhibit the fungus may retard invasion of healthy seedlings from infested soil so that the amount of late crown infection from that source is actually lessened.

The relationship of soil moisture to severity of infection seemed dependent upon host response rather than to any direct control of the fungous growth. The pathogene scarcely grew at soil moisture contents above 70 percent of capacity (table 10), but it invaded the plant most extensively at 90 percent. The fungus grew best at about 50 percent soil moisture, but the host was not invaded as severely as at 90 percent. The fungus did not progress far up the crown at 30 percent soil moisture, although it was capable of growth at soil moisture contents below this level.

The dry weight of the plants at different soil moisture contents indicated that the light infection at low soil moisture was nearly as injurious to the plants' development as the very severe infection at higher soil moisture content. Although the crown of the plant is invaded rather severely at optimum soil moisture there is little, if any, injury to the parts of the plant aboveground, and deviation in soil moisture from optimum rendered the plant subject to injury by crown infection.

The injury at high soil moisture agrees in general with the observations on seedling infection from infected seed by Holbert *et al.*

(7) and Holbert and Koehler (9). The more severe injury at low soil moisture contents is in keeping with Romyn's (15) unpublished report that seedlings from diseased seed are injured by a drop from 60 to 40 percent of the water-holding capacity when grown in sand, while disease-free plants suffer no ill effects.

No definite explanation of the reduction in growth attributable to late crown infection is available. The lower dry weight of the roots might be attributed to decay and loss of parts in washing, but the associated decrease in dry weight of the tops indicates that the chronic condition developed during the period of active growth. However, the pathogene becomes established throughout the crown so late in the developmental history of the plant that severe injury would not be expected. There is some loss of adventitious roots, and the discoloration of the xylem in the crown suggests that water consumption would be hampered, but these disabilities do not appear during the period of most active growth. As pointed out above, it is possible that the loss of the primary root system is more significant than generally assumed.

Regardless of the exact explanation, the poorer development of the infected plants is associated with a decrease in water consumption (table 12). The reduced consumption of water may be considered as either the cause or the result of the poorer growth, but the fact that mild infection at low soil moisture was more harmful than moderately severe infection at optimum moisture suggests that water was probably a limiting factor.

The reduction in growth of plants subject to late crown infection should be considered in interpreting yield data from infected plants and seed treatments. The reduction in yield from the use of *Diplodia*-infected seed [Koehler (11), Holbert, Koehler and Dungan (10), Vibar (27), Koehler and Holbert (15), Durrell (5), Raleigh (22), Melhus *et al.*, (18)] has been justly attributed to the loss of stand for the most part. However, subnormally developed plants grown from infected seed have been commonly reported [Durrell (5), Koehler and Holbert (15), Raleigh (22)], even though reduction in stand may decrease competition among plants under certain conditions sufficiently to allow an actual increase in yield per plant, according to Kiesselbach (14).

The reduction in growth due to crown infection derived from infested soil reported in the above experiments cannot be attributed to reduction in seedling vigor, as shown by Holbert *et al.* (6), for seedling infection derived from infected seed. Infection from the soil does not occur until 2 weeks after soil infestation (table 2), and the mesocotyl is never girdled before the adventitious roots are fully established. The smaller yield from individual plants weakened by *Diplodia* seedling infection than from similar plants weakened

by other means, as reported by Holbert *et al.* (6), might be due to the continued parasitism of the pathogene.

The increase in transpiration ratio is associated with a decrease in dry weight. There is an actual decrease in water consumption when crown infection is severe, but the decrease is not proportionate to the reduction in dry weight. Part of the increase in the ratio might be attributed to digestion of plant parts and plant foods. However, the increase seems to be due more likely to the disturbance of the normal balance of tops and roots, since anything that disturbs the development of the plant leads to such an increase [low fertility or limited volume of soil for corn according to Kiesselbach (13); nematode infestation of beet and celery, according to Wimmer (29); rust infection of leaves of cereals according to Murphy (20), Weiss (28), and Johnston and Miller (12), or shading of plants (21)]. The transpiration ratio is simply a measure of the plant's efficiency which would approach perfection only when the water-supplying and water-consuming parts of the plant are normally balanced.

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